Comparison of nitrogen uptake in the roots and rhizomes of *Leymus chinensis*

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Abstract

Leymus chinensis (Trin.) Tzvel is a rhizomatous grass species in the Eastern Eurasian steppe zone that is often limited by low soil nitrogen availability. Although a previous study showed that the rhizomes of *L. chinensis* have the capacity to take up nitrogen, the importance of such uptake for nitrogen nutrition is unclear. Moreover, little is known regarding the inorganic nitrogen uptake kinetics of roots and rhizomes in response to nitrogen status. Here, we first found that ammonium is preferred over nitrate and glycine for *L. chinensis* growth. Using the ^{15}N-labelling method, we found that the rate of ion influx into roots was approximately five-fold higher than into rhizomes under the same nitrogen content, and the ion influxes into roots and rhizomes under 0.05 mM N were greater than in the presence of 3 mM N, especially in the form of NH_4^+. Using a non-invasive micro-test technique, we characterised the patterns of NH_4^+ and NO_3^- fluxes in the root mature zone, root tip, rhizome mature zone, and rhizome tip following incubation in the solution with different N compounds and different N concentrations. These results suggest a dynamic balance between the uptake, utilisation, and excretion of nitrogen in *L. chinensis*.

Additional key words: ion fluxes, HATS, LATS, ^{15}N-labelling, non-invasive micro-test.

Introduction

Nitrogen is an essential element and a limiting factor in agricultural plant production. It is available to plant roots in several forms, including nitrate (NO_3^-), ammonium (NH_4^+), and organic compounds (Miller *et al.* 2007). Nitrogen fertilisers are extensively applied to increase seed production (Mengel *et al.* 2006) and forage (Kingston-Smith *et al.* 2006). However, crop plants convert only 30 - 40 % of this N to useful products; the remaining N is largely lost to the environment in gaseous form (NH_3, NO, N_2O, and N_2) or dissolved in water (NH_4^+ and NO_3^-) (Ju *et al.* 2009). These fluxes result in considerable waste of resources and environmental pollution (Liu *et al.* 2013). Therefore, it is essential to understand the physiological and molecular mechanisms of plant N uptake and utilisation to improve nitrogen-use efficiency (NUE).

Nitrogen usage is a stepwise process that includes uptake, assimilation, translocation, and remobilisation (Masclaux-Daubresse *et al.* 2010). Plant root systems have evolved specific anatomical, morphological, and physiological characteristics to absorb different nitrogenous compounds, resulting in spatial and temporal variability in the uptake of NH_4^+ and NO_3^- along plant roots (Fang *et al.* 2007, Li *et al.* 2010). The highest NO_3^- influx in *Pinus pinaster* occurs in an area of 20 - 50 mm along the root axis from the root tip (Plassard *et al.* 2002). The NO_3^- net flux near the root apex appear to be low in maize (Henriksen *et al.* 1992) but high in barley (Taylor and Bloom 1998). Persson *et al*. (2006) reported that the uptake of organic N in Scots pine is greater than or equal to that of NH_4^+, whereas NO_3^- uptake is comparatively low. Many studies have also shown that

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Abbreviations: AMTs - ammonium transport proteins; HATS - high-affinity transport system; LATS - low-affinity transport system; NMT - non-invasive micro-test; NUE - nitrogen-use efficiency.

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some species of boreal forest plants preferentially absorb NH_4^+ or amino acids over NO_3^-, even when the content of NO_3^- in soil exceeds that of NH_4^+ by as much as ten-fold (Kronzucker *et al.* 1997, Näsholm *et al.* 1998). These results suggest that the uptake of different nitrogen species by diverse plants is characterised by spatial and temporal specificity and ion selectivity.

Plants possess various kinetically distinct transport systems for the uptake of inorganic nitrogen, including the high-affinity transport system (HATS) for NO_3^- and NH_4^+ uptake and low-affinity transport system (LATS) for NO_3^- absorption (Crawford and Glass 1998, Loqueé and Von Wireé 2004). In *Arabidopsis*, the nitrate transporter 1 (NRT1) family consists of 53 members, 16 of which have been shown to transport NO_3^- with low affinity (Krapp *et al.* 2014), with the exception of NRT1.1, which works at both high- and low-affinity ranges for NO_3^- (Liu and Tsay 2003) and also functions in NO_3^- sensing (Ho *et al.* 2009). In addition, seven members of the NRT2 family encode putative HATS for NO_3^- (Liu and Tsay 2003). *Arabidopsis* ammonium transport proteins (AMTs), AtAMT1;1 to AtAMT1;5, show the greatest identity and are putative HATS for NH_4^+ (Loqueé and Von Wireé 2004). AtAMT2 represents another class of AMTs, which are homologues of the Mep1, 2, and 3 ammonium transporters of *Saccharomyces cerevisiae*, and transports NH_4^+ in an energy-dependent manner when expressed in yeast cells (Sohlenkamp *et al.* 2000). No LATS for NH_4^+ have been characterised to date in plants.

Amino acids are the primary organic nitrogen compounds, accounting for 15 - 60 % of the total nitrogen in soil (Schulten and Schnitzer 1997). Functional and homologous sequence analyses have identified a large number of potential amino acid transporters from several gene families in plant genomes, which can be classified into two major groups: the amino acid transporter family (ATF) and the amino acid polyamine choline superfamily (APC) (Rentsch *et al.* 2007, Wipf *et al.* 2002). The main subfamilies are the amino acid permeases (AAP), cationic amino acid transporters (CAT), and lysine/histidine transporters (LHT), which mediate proton-dependent import of amino acids into the cell (Elashry *et al.* 2013). In addition, some members, such as AtAAP1 and the AtAAP5-AtLHT1 double system, have been shown to mediate amino acid uptake in plant root cells (Lee *et al.* 2007, Svennerstam *et al.* 2008). Although some of these nitrogen transporters have been characterised in plants, the uptake dynamics of different nitrogen sources at the whole plant level remains unclear .

Leymus chinensis, a rhizomatous, dominant, perennial grass species, shows marked adaptability to N-deficient soils and it may have specific traits in terms of the uptake and utilisation of diverse nitrogen sources (Kang *et al.* 2007, Gao *et al.* 2008). Liu *et al.* (2011) reported that rhizomes and roots have the same ability to absorb N, although the roots of *L. chinensis* have greater N accumulation capacity. However, the importance of rhizome uptake for plant N nutrition is unclear, and there have been no reports to date regarding the inorganic N uptake kinetics of roots and rhizomes in response to nitrogen status in *Leymus* plants. In this study, we examined the effects of three forms of nitrogen on the growth of *L. chinensis*, particularly on the growth of roots and rhizomes. Then, we investigated and compared the dynamic absorption of these N forms into the roots and rhizomes of *L. chinensis* using the ^{15}N-labelling method and non-invasive micro-test (NMT) to demonstrate possible spatial and temporal variability of N fluxes.

Materials and methods

Plants and treatments: Seeds of *Leymus chinensis* (Trin.) Tzvel were obtained from the Institute of Forage and Grassland Sciences, Heilongjiang Academy of Agricultural Sciences, Heilongjiang, China. Seeds were soaked in tap water at 4 °C for 3 d and then germinated in pots containing moistened quartz sand in a greenhouse at a temperature of 25 °C, an air humidity of 50 %, a 14-h photoperiod, and an irradiance of 1 300 μmol m^{-2} s^{-1}. After 21 d, seedlings were transplanted into new pots and grown for another 21 d in the sand with Hoagland nutrient solution containing [mM]: 3 KNO_3, 0.5 NaH_2PO_4, 1.5 $Ca(NO_3)_2$, 0.1 NaFeEDTA, 2.9 × 10^{-7} $CuCl_2 \cdot 2\ H_2O$, 2.1 × 10^{-7} $CoCl_2 \cdot 6\ H_2O$, 1.8 × 10^{-6} $MnCl_2 \cdot 4\ H_2O$, 0.8 × 10^{-7} $ZnSO_4 \cdot 7\ H_2O$, 4.6 × 10^{-5} H_3BO_3, and 1.0 × 10^{-7} $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$ (the draining solution was removed).

The 45-d-old plants were randomly divided into three groups and watered with Hoagland nutrient solution or with Hoagland nutrient solution in which the KNO_3 had been replaced by either 3 mM NH_4Cl or 3 mM glycine. After growth for 28 d, all plants were separated into parent shoots, daughter shoots, rhizomes and roots, and were weighed after freeze-drying. In addition, the nitrogen content of shoots, roots, and rhizomes was determined using a *Vario EL* cube *CHNOS* elemental analyzer (*Elementat*, Langenselbold, Germany) following the method described by Nakamura *et al.* (2010).

The 21-d-old seedlings were transplanted into a black plastic basin containing full-strength Hoagland nutrition solution under a plastic cover. Plants were grown in a growth cabinet under conditions mentioned above for 6 weeks (the medium was renewed weekly). Then plants were transferred into Hoagland nutrition solution without nitrogen for 3 d and exposed to different N sources and concentrations. The nutrient solution was supplied with 3 mM or 50 μM NH_4Cl, 3 mM or 50 μM KNO_3, or 3 mM

or 50 μM glycine, respectively. After 12 h, plants were prepared for the ^{15}N-nitrogen uptake experiments and net NH_4^+ and NO_3^- flux measurements.

^{15}N-nitrogen uptake analysis: The measurement of ^{15}N-labelled nitrogen uptake was conducted according to Yuan *et al.* (2007) with minor modifications. Roots and rhizomes of plants grown in 3 mM N were rinsed with 1 mM $CaSO_4$ for 1 min, incubated for 6 min in nutrient solution containing 200 μM ^{15}N-labelled NH_4Cl, KNO_3, or glycine (95 atom% ^{15}N) and finally washed in a solution of 1 mM $CaSO_4$. For plants grown in 50 μM N, roots and rhizomes were directly incubated for 6 min in a nutrient solution containing 200 μM ^{15}N-labelled NH_4Cl, KNO_3, or glycine and washed with 1 mM of $CaSO_4$. Roots and rhizomes were harvested separately and stored at -80 °C before freeze-drying. Each sample was ground and ~1.0 mg of powder was used for ^{15}N determination by isotope mass spectrometry (*Thermo Scientific*, Suwanee, USA).

Measurements of net NH_4^+ and NO_3^- fluxes: Net fluxes of NH_4^+ and NO_3^- were measured using the non-invasive micro-test (NMT; http://www.youngerusa.com/mageflux or http://xuyue. net/mageflux) at room temperature (24 - 26 °C), as described by Sun *et al.* (2009) with modifications. Plants grown in complex nutrient solution, nitrogen-deficient solution, and solutions separately supplied with 3 mM KNO_3, NH_4Cl, or glycine after growth under nitrogen-starved conditions were selected for the ionic flux measurements. The plant underground parts, including root mature zone, root tip, rhizome mature zone, and rhizome tip, were immobilised on a small piece of quartz coverglass at the bottom of the dish and incubated in 2 - 3 cm^3 of measuring solution, 3 mM NH_4^+ or NO_3^- and 0.1 mM NH_4^+ or NO_3^-, for 10 min to equilibrate before the measurements.

The concentration gradients of the target ions were measured by moving the ion-selective microelectrode between two positions close to the plant material in a pre-set path (1 ± 0.5 μm for excised roots and rhizomes) at a programmable frequency in the range of 0.3 to 0.5 Hz. The ionic fluxes were measured slowly at ~6 s per point for 4 min. The electrode was stepped from one position to another with the computer-controlled NMT system.

Prepulled and silicified glass microelectrodes (diameter 5 ± 1 μm, *XY-DJ-02* for NH_4^+ measurement; diameter 9 ± 1 μm, *XY-DJ-02* for NO_3^-; *Younger*, Amherst, USA) were back-filled with a solution (100 mM NH_4Cl and 10 mM KNO_3) to a length of ~1 cm from the tip and front-filled with 15 to 50 μm columns of selective liquid ion-exchange cocktails (*LIX, XY-SJ-NH4* for NH_4^+ measurement; *LIX, XY-SJ-NO3* for NO_3^-, *Younger*). An Ag/AgCl wire electrode holder (*XYEH01-1*; *Xuyue Sci. and Tech. Company*, Beijing, China) was inserted in the back of the electrode to make electrical contact with the electrolyte solution. *DRIREF-2* (*World Precision Instruments*, Sarasota, USA) was used as the reference electrode. Before and after each flux measurement, the microelectrodes were calibrated with the measuring solution and the electrodes with a Nernstian slope > 56 mV per ion flux unit for NH_4^+ and >50 mV per ion flux unit for NO_3^- were used for data collection.

The net fluxes were calculated using J_{Cal} *1.0* (a free MS Excel spreadsheet, http://youngerusa.com/jcal or http://ifluxes.com/jcal). This calculation method is based on the Fick's law of diffusion: $J = -D_0 (dc/dx)$, where J is the ion flux [pmol cm^{-2} s^{-1}], D_0 is the diffusion coefficient of a specific ion in a given medium, dc is the ion concentration difference based on microvolt differences, and dx is the distance the microelectrode moved from one point to another perpendicular to the root mature zone, root tip, rhizome mature zone, or rhizome tip. Three-dimensional ionic fluxes were calculated using *MageFlux*. Trace is a recording of a typical experimental plot illustrating ion influx (negative) and efflux (positive) in five areas of plant underground parts.

Statistical analysis: Data are presented as means ± SEs. Each measurement was performed in triplicate (10 - 12 seedlings per measurement). All data were statistically analysed using the software package *SPSS* (for *Windows*, *v. 13.0*). Significant differences are indicated by different letters (Fisher's two-independent-samples test, $P < 0.05$).

Results

To explore the effects of different forms of nitrogen on the growth of *L. chinensis* plants, we investigated the dry mass (DM) and total N content of shoots, roots, and rhizomes in the presence of 3 mM NH_4^+, NO_3^-, and glycine. We found that the DM and N content of the parent shoots were almost two-fold greater than those in daughter shoots under different N sources. The DM and total N content of parent and daughter shoots were higher in solution with 3 mM NH_4^+ than with 3 mM NO_3^- or 3 mM glycine, and there was no significant difference in plant growth between the glycine and NO_3^- (Fig. 1*A*,*B*). We further found that the DM and total N content of roots and rhizomes at the NH_4^+ treatment were two-fold those at the glycine and NO_3^- treatments (Fig. 1*C*,*D*). These results revealed that NH_4^+ is preferable for the growth of *L. chinensis* plants compared to NO_3^- and organic N.

To evaluate the nitrogen uptake capacities of the roots and rhizomes of *L. chinensis*, short-term influx of three ^{15}N-labelled nitrogen species was determined in the presence of different concentrations of N. In plants supplied with a high concentration of N, *i.e.*, 3 mM NH_4Cl, KNO_3, or glycine, there was no significant

difference in ion influx into roots and rhizomes between KNO_3 and NH_4Cl, but the glycine influx was lower (Fig. 2*A*,*B*). However, in plants supplied with low concentration of N, *i.e.*, 50 μM NH_4Cl, KNO_3, or glycine, NH_4^+ influx into roots and rhizomes was greatest, followed by NO_3^-, and glycine influx was lowest (Fig. 2*C*,*D*). More interestingly, the N uptake capacity of roots and rhizomes was higher when supplied with a low than a high concentration of N. In addition, rhizomes had the ability to take up three different N compounds but exhibited almost a five-fold lower ion influx than roots for the same N compound.

We further used NMT to assess the pattern of NH_4^+

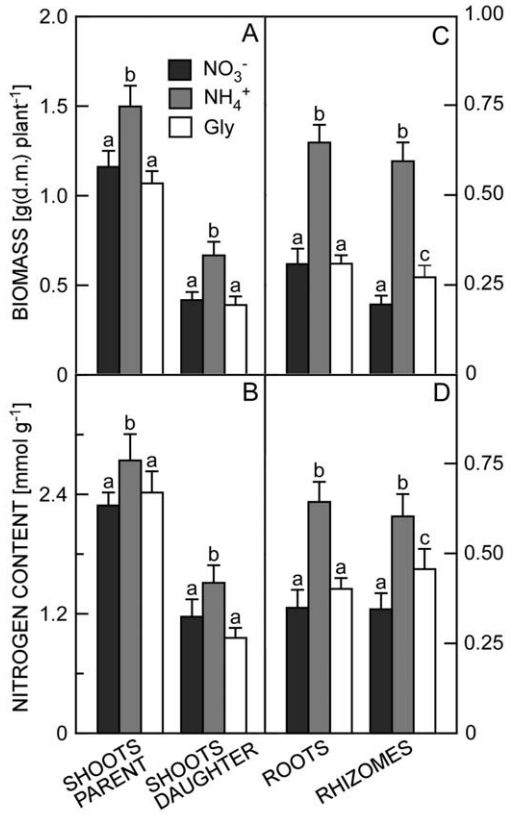

Fig. 1. Biomass and nitrogen content of *L. chinensis* after culture at 3 mM NO_3^-, 3 mM NH_4^+, or 3 mM glycine for 76 d. *A* - biomass of parent and daughter shoots; *B* - total N content of parent and daughter shoots; *C* - biomass of the roots and rhizomes; *D* - total N content of the roots and rhizomes. Each measurement was performed in triplicate (65 - 70 seedlings per measurement). Means ± SEs. Significant differences at $P < 0.05$ are indicated by different letters.

fluxes in two root sites, the root mature zone and root tip, and two rhizome sites, the rhizome mature zone and rhizome tip. We found that 3.0 mM NH_4^+ flux was inward in these four sites and the influx values in the root mature zone, root tip, and rhizome tip under N-deficient conditions were higher than those under N-sufficient conditions, however, the values in the rhizome mature zone showed the opposite pattern (Fig. 1*A* Suppl.). The rhizome tip displayed maximal influx of -383 pmol cm^{-2} s^{-1} under N-sufficient conditions and -847 pmol cm^{-2} s^{1} under N-deficient conditions (Fig. 3*A*). The 0.1 mM NH_4^+ fluxes in the four sites were mostly inward under N-sufficient and N-deficient conditions, although that in the rhizome mature zone was outward under N-deficient conditions (Fig. 1*B* Suppl.). The influx of 0.1 mM NH_4^+ in the root mature zone and rhizome tip was higher under nitrogen-deficient conditions than under N-sufficient conditions, which was similar to the case of 3.0 mM NH_4^+ in the same sites. The maximal influx of 0.1 mM NH_4^+ was -258 pmol cm^{-2} s^{-1} in root tips under N-sufficient conditions (Fig. 3*B*). These results showed that NH_4^+ was taken up by underground parts of *L. chinensis* plants under N-sufficient and N-deficient conditions, except that 0.1 mM NH_4^+ was released by the rhizome mature zone under N-deficient conditions.

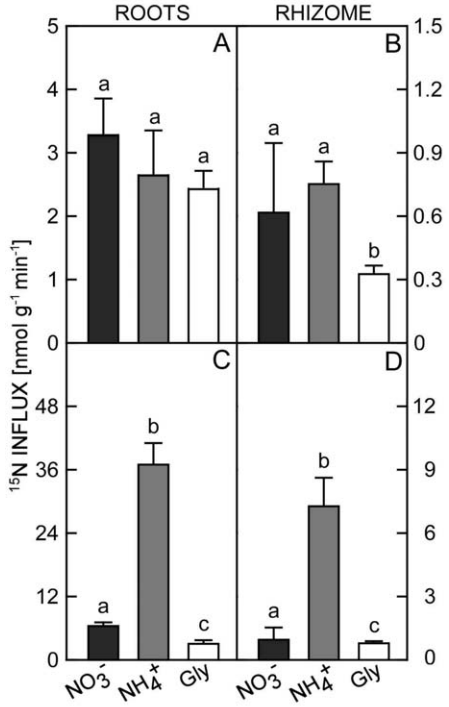

Fig. 2. The uptake of ^{15}N-labelled nitrogen into roots and rhizomes of plants after resupply with 3 mM NO_3^-, NH_4^+, or glycine (*A*,*B*) or 50 μM NO_3^-, NH_4^+, or glycine (*C*,*D*) for 12 h. Means ± SEs (n = 6 - 8 plants). Significant differences at $P < 0.05$ are indicated by different letters

To compare the NH_4^+ fluxes at roots and rhizomes between N-starved conditions and after the resupply of 3 mM NH_4^+, NO_3^-, or glycine, the 3 and 0.1 mM NH_4^+ fluxes were measured using the NMT technique. We found that the 3 mM NH_4^+ fluxes in these four sites were inward in three N-resupplied media, which was the same as under the N-starved conditions (Fig. 4*A*). In the NO_3^--resupplied culture, the 3 mM NH_4^+ influx increased in the three sites and the maximum in the root mature zone and rhizome mature zone was three-fold higher than

under N-deficient conditions, with the exception of the NH_4^+ influx into the root tip, which was decreased to ~20 % under N-deficient conditions. In the NH_4^+-resupplied condition, the 3 mM NH_4^+ influx

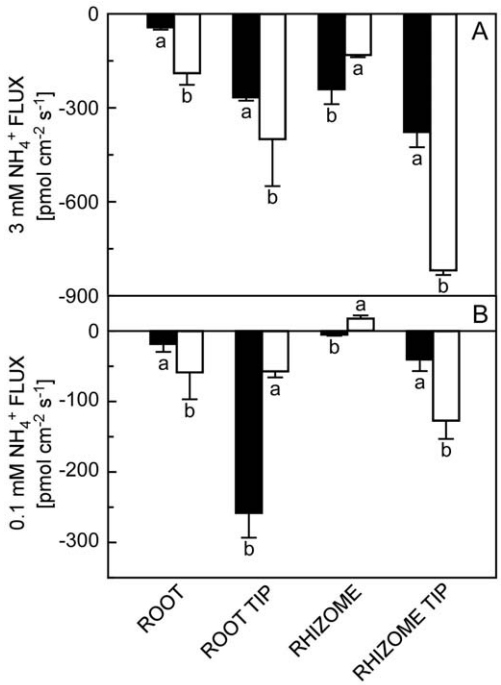

Fig. 3. The net NH_4^+ fluxes in different parts of the root and rhizome under N-sufficient (*black*) or N-deficient (*white*) conditions. *A* - 3 mM NH_4^+ fluxes, *B* - 0.1 mM NH_4^+ fluxes. Means ± SEs (n = 4). Significant differences at $P < 0.05$ are indicated by different letters.

decreased in three sites, the exception being the root mature zone. The minimum value in the rhizome tip was ~10 % of that under the N-deficient condition and the maximum influx in the root mature zone was increased by 1.8-fold. Under the glycine-resupplied condition, the 3 mM NH_4^+ influx increased in the root mature zone and rhizome mature zone, and decreased in the root tip and rhizome tip. The maximum NH_4^+ influx was 2.4-fold higher in the root mature zone, and the values in the root tip and rhizome tip were almost identical, being ~60 % of those under N-deficient conditions. Furthermore, we found that the 0.1 mM NH_4^+ net flux was inward under 3 mM NO_3^--resupplied conditions and outward under 3 mM NH_4^+- and glycine-resupplied conditions in these four sites, with the exception of influx in the rhizome tip under 3 mM NH_4^+-resupplied conditions (Fig. 4*B*).

Net NO_3^- fluxes were characterised in the same four sites under N-sufficient and N-deficient conditions, similarly to the NH_4^+ flux measurement. The 3 mM and 0.1 mM NO_3^- fluxes were outward in all four sites under both N-sufficient and N-deficient conditions, and the flux values were lower under N-deficient conditions than under N-sufficient conditions in all four sites (Fig. 2 Suppl.). The root tip displayed a maximal efflux of 3 mM NO_3^- (1076 pmol cm^{-2} s^{1}) under N- sufficient conditions and the rhizome mature zone displayed a maximal efflux of 0.1 mM NO_3^- (270 pmol cm^{-2} s^{1}) under N-deficient conditions. Moreover, the maximum 3 mM NO_3^- flux was 99 pmol cm^{-2} s^{-1} in the root mature zone and the maximum 0.1 mM NO_3^- flux was 41 pmol cm^{-2} s^{-1} in the rhizome mature zone under N-deficient conditions (Fig. 5).

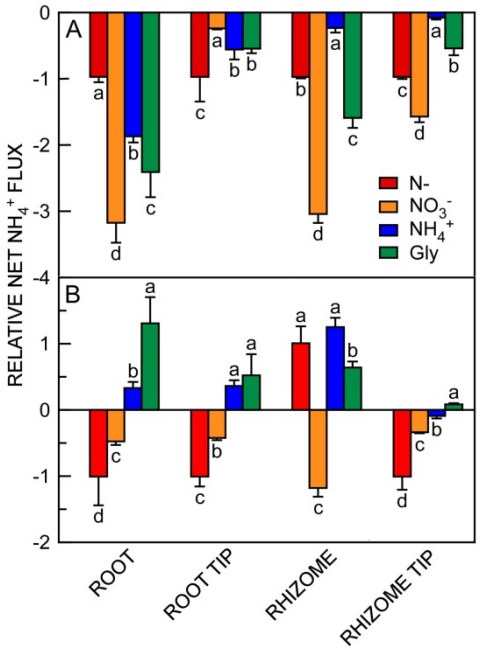

Fig. 4. The Relative net NH_4^+ fluxes in different parts of roots and rhizomes under N-starvation (*red*) or after resupplied with NH_4^+ (*blue*), NO_3^- (*orange*), or glycine (*green*). *A* - 3 mM NH_4^+ net flux profiles, *B* - 0.1 mM NH_4^+ net flux profiles. The data at each point were collected every 6 s during a 4 min recording. The NH_4^+ influx under N-starved conditions is shown as -1. Means ± SEs (n = 4). Significant differences at $P < 0.05$ are indicated by different letters.

We further compared the pattern of 3 mM and 0.1 mM NO_3^- flux under N-resupplied conditions compared with N-deficient conditions. In the NO_3^--resupplied medium, the 3 mM NO_3^- fluxes were inward in all four sites, which was the converse of the NO_3^- flux under N-deficient conditions. In addition, under NH_4^+-resupplied conditions, the 3 mM NO_3^- flow was outward in the root mature zone and root tips, and inward in the rhizome mature zone and rhizome tips. However, in the glycine-resupplied conditions, the 3 mM NO_3^- flow was outward in the root mature zone and rhizome mature zone, and inward in the root tips and rhizome tips (Fig. 6*A*).

We further found that the 0.1 mM net NO_3^- flux was diverse in the four sites under identical N-resupplied conditions (Fig. 6*B*). Under NO_3^--resupplied conditions, the 0.1 mM NO_3^- flux was inward in the root mature zone and rhizome mature zone, and outward in the root tip and

rhizome tip. Under the NH_4^+-resupplied condition, the 0.1 mM NO_3^- flux was inward in the root mature zone and outward in the root tip, rhizome mature zone, and rhizome tip. It is interesting that the 0.1 mM NO_3^- flux was outward in all four sites under the glycine-resupplied condition, and the maximum value in the root mature zone was ~15-fold higher than that under N-deficient conditions. These results revealed that the transient fluxes of NH_4^+ and NO_3^- exhibited a region-specific and N-concentration dependent pattern in the plant underground parts enabling *L. chinensis* plants to adapt to N-deficient soil.

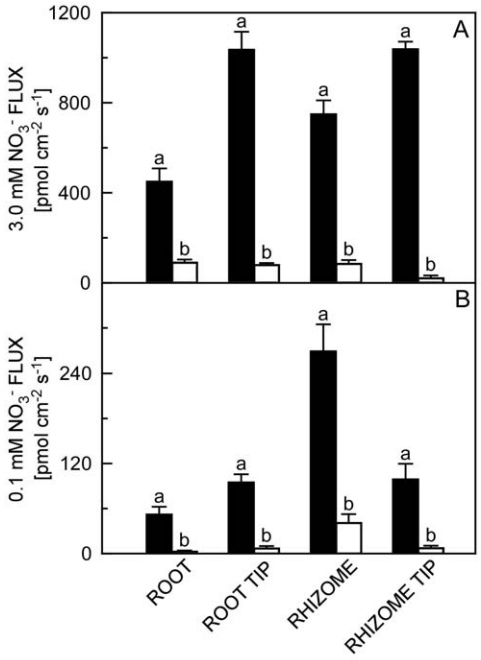

Fig. 5. The net NO_3^- fluxes in different parts of the root and rhizome under N-sufficient (*black*) or N-deficient (*white*) conditions. *A* - 3 mM NO_3^- fluxes, *B* - 0.1 mM NO_3^- fluxes. Means ± SEs (n = 4). Significant differences at $P < 0.05$ are indicated by different letters.

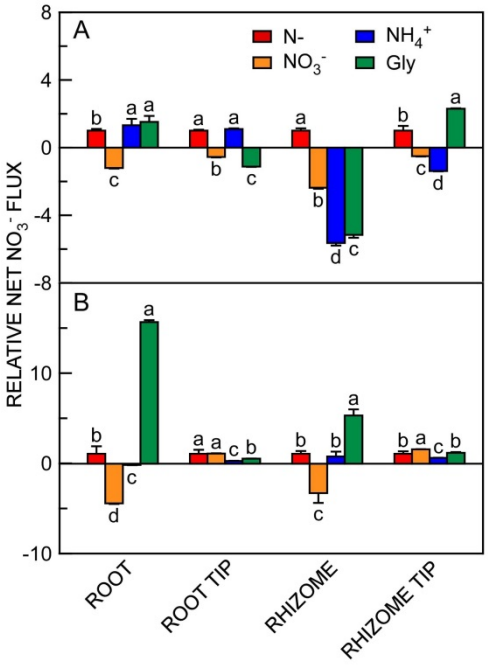

Fig. 6. Relative net NO_3^- fluxes into roots and rhizomes under N-starvation (*red*) and after resupplied with NH_4^+ (*blue*), NO_3^- (*orange*), or glycine (*green*). *A* - 3 mM NO_3^- net flux profiles, *B* - 0.1 mM NO_3^- net flux profiles. The data at each point were collected every 6 s during a 4 min recording. The NO_3^- flux under N-starvation is shown as 1. Means ± SEs (n = 4). Significant differences at $P < 0.05$ are indicated by different letters.

Discussion

Leymus chinensis is a clonal rhizomatous dominant grass species in the Eastern Eurasian Steppe zone, the growth of which is frequently limited by low soil nitrogen availability. In this study, we evaluated the effects of different nitrogen sources on the growth of *L. chinensis* and determined the NH_4^+ and NO_3^- fluxes in two sites of plant roots (mature zone and tip) and two sites of rhizomes (mature zone and tip) under high and low N supply. The net NH_4^+ and NO_3^- flux patterns in the roots and rhizomes were complex, and showed site-specific and concentration-dependent characteristics, indicating that roots and rhizomes play different roles in nitrogen usage and facilitate *L. chinensis* growth in N-deficient soil.

Plants are unable to accumulate high content of NH_4^+ because it becomes toxic (Walch-Liu *et al.* 2001). The symptoms of NH_4^+ toxicity might be: leaf chlorosis, growth suppression, yield depression, and cell death (Britto and Kronzucker 2002). Much research has been directed toward unravelling the causes of NH_4^+ toxicity and mechanisms of NH_4^+ tolerance in plants, which include cellular pH change, ionic balance, relationships with carbon metabolism, phytohormone responses and bioenergetics of primary NH_4^+ transport (Britto and Kronzucker 2002). Chen *et al.* (2013a) showed that a high-NUE rice cultivar Wuyunjing 23 (W23) possesses greater capacity to resist NH_4^+ toxicity than a low-NUE cultivar Guidan 4 (GD), due to NH_4^+ efflux in the root elongation zone of W23 being almost sevenfold lower than that in GD, which was coupled to strongly stimulated root respiration. In our study, the biomass and total nitrogen content of shoots, roots, and rhizomes of *L. chinensis* in the presence of 3 mM NH_4^+ were significantly higher than those under 3 mM NO_3^- and

glycine. These findings indicate the existence of specific mechanisms for adjusting cellular NH_4^+ content, which vary in response to the uptake of external NH_4^+ and the release and assimilation of intracellular NH_4^+ in this plant.

Leymus chinensis has a rhizome, which links the individual ramets to generate extensive rhizome systems and accounts for a significant proportion of the underground parts of the plant. The rhizome of sedge *Carex bigelowii* also absorb nitrogen upon the direct application of $^{15}NH_4{}^{15}NO_3$ (Brooker *et al.* 1999). In this study, we showed that N absorption by the rhizome was markedly lower than that by roots in the presence of high and low N supply, which is similar to the results of Liu *et al.* (2011). Furthermore, we demonstrated that rhizomes of *L. chinensis* could directly absorb three different nitrogenous compounds, and exhibited diverse uptake efficiencies and capacities in comparison with roots, which may contribute to the dominance of *L. chinensis* in N-deficient environments.

Plants are capable of utilising different N compound as the sole N source. Nitrate is transported into the cell *via* nitrate transporters and then reduced by nitrate reductase to nitrite, which is converted to NH_4^+ (Cabrera *et al.* 2014). Next, NH_4^+ can be used during synthesis of amino acids, *e.g.* glutamine *via* glutamine synthetase (Meng *et al.* 2016). In general, plant NUE is dependent on N-uptake and N-utilisation (Han *et al.* 2015). In ^{15}N-labelled N uptake experiments, we found no marked differences in uptake of the three N compounds into roots and rhizomes in the presence of high N supply. However, the plants showed more effective N-uptake at low N supply, and the NUE was higher at low concentrations of NH_4^+ compared to NO_3^- and glycine. These results indicated that the HATS-mediated influx of N is more efficient than that mediated by LATS in *L. chinensis*.

Using the NMT method, we found that the net flux of NH_4^+ was inward at four sites under N-sufficient and N-deficient conditions, with the exception of an outward 0.1 mM NH_4^+ flux in the rhizome mature zone under N-deficiency, which indicated that the rhizome functions as a large sink for N and provides plants with the capacity to take advantage of transient nutrient supplies and compensate in part for low nutrient supply, in agreement with a previous report of Brooker *et al.* (1999). Plants can sense the extracellular N content and induce the expression of NH_4^+ transporters, such as *AtAmt1;1*, *AtAmt1;2*, *AtAmt1;3*, *AtAmt1;5* and *AtAmt2;1* (Loqueé and Von Wireé 2004; Yuan *et al.* 2007), and NO_3^- transporters; *i.e.*, NRT2.1, NRT2.2 and NRT2.4, in *Arabidopsis* (Kiba *et al.* 2012), which may explain the increase in NH_4^+ net influx and decrease in net NO_3^- efflux after N starvation. Therefore, *L. chinensis* transcriptome databases (Chen *et al.* 2013b, Sun *et al.* 2013) can facilitate determination of the molecular mechanism of nitrogen uptake and assimilation and provide potential target genes for the molecular breeding of *Leymus* and other species.

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